

A Molecular Phylogenetic Study of *Lonicera* L. (Caprifoliaceae) in Japan Based on Chloroplast DNA Sequences

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Despite previous phylogenetic studies, the relationships among sections, subsections and species of *Lonicera* (Caprifoliaceae), especially in Japan, remain unclear. In this context, we investigated the phylogenetic relationships among 23 Japanese species belonging to all four sections and five subsections of *Lonicera* based on sequences of five chloroplast non-coding regions, *rpoB-trnC*, *atpB-rbcL*, *trnS-trnG*, *petN-psbM*, *psbM-trnD*. The trees obtained from were fundamentally identical with those reported previously, but provide some new insights on the Japanese species. 1) All Japanese species of *Lonicera* concerned here are assignable to one clade, corresponding to subgenus *Lonicera* sensu Hara. 2) Both sections *Caeruleae* and *Lonicera* are monophyletic, while neither section *Nintooa* nor *Ishika* is monophyletic. 3) Within section *Ishika*, each of the four subsections, *Ishika*, *Monanthae*, *Bracteatae* and *Rhodanthae* forms a clade, although the branch supports for certain clades are weak. 4) Subsection *Purpurascetes* is a polyphyletic group. Two Japanese species, *Lonicera ramosissima* and *L. linderifolia* form a clade with weak support, but the other species of subsection *Purpurascetes* distributed in China form the other clade. 5) Subsection *Rhodanthae* is more closely related to sections *Nintooa* and *Lonicera* than to other subsections. Based on the results, the circumscription of the higher taxonomic groups for the Japanese species of *Lonicera* proposed by Hara in 1983 is fundamentally acceptable.

Kew Words: Caprifoliaceae, chloroplast non-coding region, *Lonicera*, phylogenetic study

Lonicera L. (Caprifoliaceae), comprising approximately 180 species of deciduous or evergreen shrubs and woody climbers, is the largest genus in the Caprifoliaceae (Mabberley 2008). It is largely distributed in temperate to subtropical regions of the northern hemisphere: Europe, Russia, eastern Asia and North America (Hsu & Wang 1988, Mabberley 2008). Since Linnaeus (1753) established *Lonicera*, the genus has historically posed various systematic problems, especially in its infrageneric classification. Maximowicz (1877) was the first to propose a comprehensive system for *Lonicera* of Asia and subdivided the genus into three subgenera, *Caprifolium*

(Tourn.) Maxim., *Chamaecerasus* (Tourn.) Maxim. and *Xylostron* (Tourn.) Maxim. Subsequently, Rehder (1903, 1913) classified *Lonicera* into the subgenera *Chamaecerasus* (L.) Rehder (including *Xylostron* sensu Maximowicz) and *Periclymenum* (L.) Rehder (= *Caprifolium*). In Rehder's system, subgenus *Chamaecerasus* was further subdivided into four sections and 20 subsections. Subgenus *Periclymenum* was subdivided into four subsections. Following Rehder, Hsu & Wang (1988) proposed a new system for the Chinese species of *Lonicera*. They divided the genus into two subgenera, *Chamaecerasus* and *Lonicera* (changed from *Periclymenum* sensu Rehder), and

rejected some of Rehder's subsections. Additionally, they recognized four sections and 12 subsections within subgenus *Chamaecerasus* in China. Yang *et al.* (2011), however, did not recognize taxa at the section level in their treatment in the Flora of China. Nakai (1938) assigned the Japanese species of *Lonicera* to 15 sections and 8 subsections under the genus *Lonicera*. Later, Hara (1983) improved Nakai's system for the Japanese species, following Maximowicz and Rehder, and classified them into two subgenera, *Lonicera* and *Caprifolium* (Mill.) Dippel, four sections, *Isika* (Anderson) Rehder, *Caeruleae* (Rehder) Nakai, *Lonicera* and *Nintooa* (Sweet) Maxim. and five subsections (Table 1). Ohba (1993) fundamentally followed Hara (1983) and recognized four sections within *Lonicera* in the Flora of Japan.

A recent phylogenetic study of the Caprifoliaceae and *Lonicera* by Theis *et al.* (2008), based on nuclear and chloroplast DNA sequences, clarified many systematic problems at the generic and subgeneric levels. Additionally, Theis *et al.* (2008) showed the monophyly of subgenera *Lonicera* (= *Chamaecerasus*) and *Caprifolium* (= *Periclymenum*), and provided insights into the relationships among sections. The relationships among and within the sections, however, still remain unclear, especially for the Japanese species. Theis *et al.* (2008) analyzed the phylogenetic relationships of 47 species of *Lonicera* in the northern hemisphere, but only 7 species were from Japan. Twenty four species of *Lonicera* occur in Japan, and 17 species of them are endemic (H. Hara 1983, 1989, Ohba 1993, Kadota 2001). The circumscription of the taxonomic groups and phylogenetic relationships of the endemic species have been poorly understood.

To clarify the phylogenetic relationships among the sections, subsections and species of *Lonicera*, we constructed phylogenetic trees using 67 species, including 22 species, 16 varieties, and 4 forms from Japan, 45 species from INSD (International Nucleotide Sequence Database). The phylogenetic relationships were constructed based on sequences of five chloroplast non-coding regions, *rpoB-trnC*, *atpB-rbcL*, *trnS-trnG*, *petN-psbM*, *psbM-trnD*. Our goals were to 1) as-

sess the circumscription of sections and subsections as proposed by Hara (1983), 2) to clarify the relationships among and within sections, especially for Japanese the species, and 3) to clarify the evolutionary relationships of the endemic species.

Materials and Methods

Plant materials and outgroup selection

We collected 81 samples representing 30 species of *Lonicera* in Japan, South Korea, China and Nepal (Appendix 1). In Japan, there are 24 species, 20 varieties, and 14 forms (H. Hara 1983, 1989, Ohba 1993, Kadota 2001). Among them we examined 22 species, 16 varieties and 4 forms. Some samples were from specimens deposited in the herbarium or from plants cultivated in botanical gardens (Appendix 1). Leaf tissues were dried with silica gel. Voucher specimens will be deposited in the Makino Herbarium (MAK), Tokyo Metropolitan University, Tokyo, the Herbarium of the University of Tokyo (TI), Tokyo, or the Herbarium of the National Science Museum (TNS), Tsukuba.

According to recent phylogenetic analyses of *Lonicera* and allied genera based on nuclear and chloroplast DNA sequences, *Lonicera* is closely related to *Leycesteria* Wall., *Symphoricarpos* Duhamel and *Triosteum* L., and is sister to the former two genera (Donoghue *et al.* 2003, Bell 2004, Theis *et al.* 2008). We therefore selected these three genera as outgroups for the phylogenetic analysis (Appendix 1). We also selected 45 species of *Lonicera* used previously by Theis *et al.* (2008) to compare with the Japanese species (Appendix 1).

DNA extraction, PCR amplification and sequencing

DNA was extracted from silica gel dried leaves using a slightly modified cetyl trimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1987). Five chloroplast noncoding regions, *trnS-trnG* (Hamilton 1999), *atpB-rbcL* (Zurawski *et al.* 1984, Crayn & Quinn 2000), *rpoB-trnC*

TABLE 1. Systematic arrangements of *Lonicera* by Maximowicz (1877), Rehder (1903, 1913), Nakai (1938), Hara (1983) and Hsu & Wang (1988).

Maximowicz (1877)	Rehder (1903, 1913)	Nakai (1938)	Hara (1983)	Hsu and Wang (1988)
Subgenus <i>Xylostron</i>	Subgenus * <i>Chamaecerasus</i>		Subgenus * <i>Lonicera</i>	Subgenus <i>Chamaecerasus</i>
	Sect. <i>Isoxylosteum</i> Subsect. <i>Microstylae</i> Subsect. <i>Spinosae</i>	Sect. <i>Isoxylosteum</i>		Sect. <i>Isoxylosteum</i> Subsect. <i>Cupulatae</i>
	Sect. <i>Isika</i> Subsect. <i>Coeruleae</i>	Sect. <i>Caeruleae</i>	Sect. <i>Caeruleae</i>	Sect. <i>Isika</i> Subsect. <i>Caeruleae</i>
	Subsect. <i>Purpurascens</i>	Sect. <i>Ramosissimae</i> Sect. <i>Parahodanthae</i>	Sect. <i>Isika</i> Subsect. <i>Purpurascens</i>	Subsect. <i>Purpurascens</i>
	Subsect. <i>Alpigenae</i> Subsect. <i>Cerasinae</i> Subsect. <i>Pileatae</i> Subsect. <i>Chlamydocarpi</i> Subsect. <i>Vesicariae</i> Subsect. <i>Bracteatae</i>	Sect. <i>Macrospemae</i> Subsect. <i>Aloigenae</i> Subsect. <i>Cerasinae</i>	Subsect. <i>Monanthae</i> Subsect. <i>Isika</i>	Subsect. <i>Alpigenae</i> Subsect. <i>Pileatae</i> Subsect. <i>Chlamydocarpi</i>
		Sect. <i>Vesicaria</i> Sect. <i>Bracteatae</i> Subsect. <i>Eubracteatae</i> Subsect. <i>Paramosissimae</i>	Subsect. <i>Bracteatae</i>	Subsect. <i>Bracteatae</i>
		Sect. <i>Praeflorentes</i>		
	Subsect. <i>Fragrantissimae</i> Subsect. <i>Pyrenaicae</i> Subsect. <i>Distegiae</i> Subsect. <i>Oblongifoliae</i> Subsect. <i>Rhodanthae</i>	Sect. <i>Fragrantissimae</i>	Subsect. <i>Rhodanthae</i>	Subsect. <i>Fragrantissimae</i> Subsect. <i>Rhodanthae</i>
		Sect. <i>Rhodanthae</i> Subsect. <i>Eurhodanthae</i> Subsect. <i>Terrameriae</i>		
Subgenus * <i>Chamaecerasus</i>	Sect. * <i>Coeloxyloteum</i> Subsect. * <i>Ochranthae</i> Subsect. <i>Tataricae</i>	Sect. * <i>Ochranthae</i> Subsect. <i>Euochranthae</i> Subsect. <i>Subsessiliflorae</i>	Sect. * <i>Lonicera</i>	Sect. <i>Coeloxyloteum</i> Subsect. <i>Ochranthae</i>
Subgenus <i>Caprifolium</i>	Sect. <i>Nintooa</i> Subsect. <i>Calcaratae</i> Subsect. <i>Breviflorae</i> Subsect. <i>Longiflorae</i>	Sect. <i>Nintooa</i>	Sect. <i>Nintooa</i>	Sect. <i>Nintooa</i> Subsect. <i>Calcaratae</i> Subsect. <i>Volubilis</i>
	Subgenus <i>Periclymenum</i> Subsect. <i>Cypheolae</i> Subsect. <i>Phenianthi</i> Subsect. <i>Eucarpifolia</i> Subsect. <i>Thoracianthae</i>		Subgenus <i>Caprifolium</i>	Subgenus ** <i>Lonicera</i> Subsect. ** <i>Lonicera</i> Subsect. <i>Phenianthi</i>

*: Taxa which include type of *Lonicera* (*L. xylosteum*, designated by Hara 1983) should be named "*Lonicera*" as autonym.**: *Lonicera caprifolium* is recognized as type, but it is not reasonable.

(Shaw *et al.* 2005), *psbM-trnD* (Lee & Wen 2004) and *petN-psbM* (Lee & Wen 2004), were used in this study. Polymerase chain reactions (PCR) for all regions were conducted in a total volume of 20 μ L, consisting of 1.0 unit of Nova Taq, 10 μ L of 2x Ampdirect Buffer with dNTP (Shimazu Bio, Japan), 8 μ L of each 10pM primer, 10–30 ng of genomic DNA. The PCR product was cleaned with 0.15 μ L ExoSap-IT enzyme (GE Healthcare) and 2.85 μ L of Milli-Q water (Millipore). Incubation was done at 37°C for 20 minutes and subsequently at 85°C for 30 minutes for deactivation of the enzyme. Sequence reactions were prepared using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The reaction mixtures were analyzed on ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were edited in Chromas Pro version 1.34 (Technelysium Pty Ltd., Tewantin, Queensland, Australia). The sequences obtained were submitted to INSD (accession numbers are shown in Appendix 1).

Alignment

The edited sequences were aligned by MAFFT (Katoh *et al.* 2002). Aligning parameters were default settings. To remove all positions with gaps the aligned sequences were trimmed by TRIMAL 1.2 (Capella-Gutierrez *et al.* 2009) with the option 'nogaps'.

Phylogenetic analyses

We conducted a maximum parsimony analysis of all five sequences as separated data using PAUP* v4.0 b10 (Swofford 2003). Heuristic searches with the tree-bisection-reconnection (TBR) branch swapping option and 10,000 random addition replicates were used. The Bootstrap test was applied with 10,000 repeats using heuristic searches to estimate support for individual clades (Felsenstein 1985).

We also conducted maximum likelihood and Bayesian analyses. The maximum likelihood tree was constructed with RAxML software (Stamatakis 2006). Bayesian analysis was performed by MrBayes version 3.12 (Huelsenbeck & Ronquist

2001). Before phylogenetic analysis of the five regions, the homogeneity of the nucleotide composition was tested among all operational taxonomic units (OTUs), since it is known that compositional heterogeneity biases phylogenetic inferences (Jermiin *et al.* 2004). Because no compositional heterogeneity was found among the regions, we combined all regions and constructed the phylogenetic trees using the combined data.

A base substitution model for each region was selected using the Akaike information criterion (AIC; Akaike 1974) from software Kakusan4 (Tanabe 2007). The nucleotide substitution model GTR + Γ was chosen in all loci for maximum likelihood and Bayesian methods (Table 2). In model selection among nonpartitioned, proportional and separate models, the proportional model was chosen for RAxML, while the non-partitioned model was chosen for MrBayes (Table 2). For Maximum likelihood analysis, the shotgun search algorithm was applied in a tree searching strategy, and 1,000 repeat bootstrap tests were conducted using a maximum likelihood algorithm. In the Bayesian analysis, two independent runs of eight chains of Markov Chain Monte Carlo (MCMC) were examined for 30,000,000 generations and sampled every 1000 generations. Two independent runs were compared to assess convergence. The average of the standard deviation of the split frequencies was less than 0.01. In addition, the effective sample size (ESS) of all parameters was calculated by Tracer version 1.5 (Rambaut & Drummond 2009). We checked whether all ESS parameters were greater than 300 or not. The first 10,000 trees were discarded as burn-in. The remaining trees from each run were combined and were used to perform a 50% majority rule consensus tree, and the posterior probabilities (PP) of their trees were calculated.

Results

Sequence characteristics

The sequence information for five chloroplast non-coding regions, *rpoB-trnC*, *atpB-rbcL*, *trnS-*

trnG, *petN-psbM*, *psbM-trnD*, including the length of each region, number of parsimony informative sites (PI), consistency index (CI), retention index (RI) is summarized in Table 2. The total aligned lengths were 5,663 bp; sequence lengths without insert/deletion positions were 2,463 bp. Total parsimony informative characters were 280. Previously, Theis *et al.* (2008) reported that several species of *Lonicera* from Japan and eastern Asia had large deletions in the sequences of two loci: *trnS-trnG* and *rpoB-trnC*. In our study, we also found that a few samples (*L. bournei*, *L. japonica* 2, 6, see Appendix 1) had large gaps in the following two loci: *petN-psbM* and *psbM-trnD*. The aligned lengths of these two loci were over 1,200 bp, but lengths excepting gaps were less than 300 bp (Table. 2). In our study, we excluded the gaps from the phylogenetic analysis. The phylogenetic analyses resulted in identical trees whether taxa with large gaps (over 50% in aligning length per each locus) were included or not.

Phylogenetic analyses

Phylogenetic analyses of maximum parsimony, maximum likelihood and Bayesian strategy based on the sequences of five regions without gaps (total 2,463 bp) were conducted. We found a maximum likelihood tree with RAxML (Best tree score was -7910.531). This ML tree is shown in Figure 1 with ML and MP bootstrap percentages and Bayesian posterior probabilities above the branches. The Bayesian tree was similar to the ML tree. Most branches were supported by high score of Bayesian posterior probability (PP=1.00, tree results not shown). Maximum parsimony analysis of the five combined regions resulted in 130 most parsimonious trees, each of 603 steps. These trees had a consistency index (CI) of 0.789 and a retention index (RI) of 0.911. The strict consensus tree is shown in Figure 2.

In all analyses, *Lonicera* was resolved as a single clade (Figs. 1, 2) divided into two clades. The two clades, corresponding to subgenera *Lonicera* and *Caprifolium*, were supported with high bootstrap percentages (100%) of ML and MP and with high Bayesian posterior probability

(PP=1.00), in agreement with the previous phylogenetic study of Theis *et al.* (2008). Also, the results showed that all Japanese species of *Lonicera* concerned here are assignable to subgenus *Lonicera* (Fig. 1). Within this clade, the Japanese species are scattered among several clades, and were often nested within some species groups distributed in northeastern Asia.

In the phylogenetic tree obtained (Figs. 1, 2), *Lonicera morrowii* from Japan formed a clade with *L. tatarica*, *L. sovetkinae* and *L. korolkowii* of northeast to central Asia. *Lonicera demissa* and *L. kurobushiensis* both endemic to Japan, formed a clade with *L. koehneana* from China and *L. xylosteum* from Europe with high branch support (MP, ML bootstrap > 90%, PP=1.00). *Lonicera maackii* distributed widely in northeast Asia and well known as an endangered species in Japan, formed a clade with *L. ruprechtiana* and *L. subsessilis* with high support (MP and ML bootstrap > 95%, PP=1.00; Fig. 1). *Lonicera uzenensis* distributed only in the Tohoku District of Honshu, Japan, was assigned to the same clade as *L. maximowiczii* and *L. conjugialis*. *Lonicera mochidzukiana* was also closely related to these three species, but its branch support was weak. In the phylogenetic tree based on the dataset excluding *L. bournei* and *L. japonica* (sample no. 2, 6, see Appendix 1) with large deletions, two samples of *L. mochidzukiana* were monophyletic and their branch support, including *L. uzenensis*, *L. maximowiczii* and *L. mochidzukiana*, was improved (ML bootstrap > 80, BP =1.0). *Lonicera tschonoskii* formed a clade with the above three species but its support was weak. *Lonicera cerasina* endemic to western Japan, formed a monophyletic group with *L. vidalii*, *L. alpigena* and *L. hemsleyana*. *Lonicera linderifolia* and *L. ramossissima*, both endemic to Japan, were sister to each other, but their branch support was weak (MP, ML bootstrap < 75%, PP=0.96). *Lonicera praeflorens* and *L. strophiphora* formed a clade that was sister to a clade of *L. harae*, *L. fragrantissima* and *L. hispida*. Samples of *L. caerulea* formed a clade. This clade was sister to a clade of infraspecific taxa of *L. gracilipes* in the ML tree (Fig. 2), but its branch support was weak.

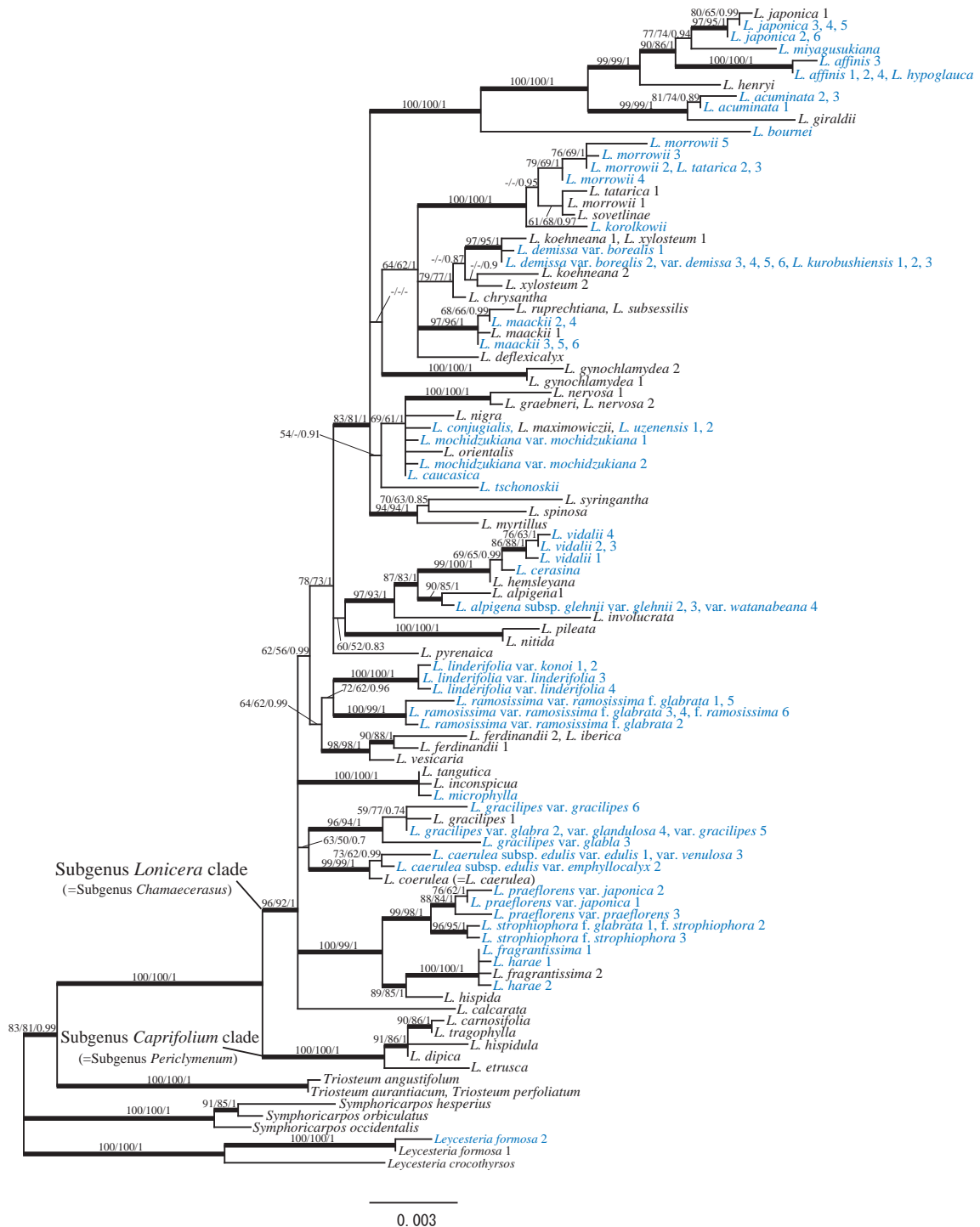
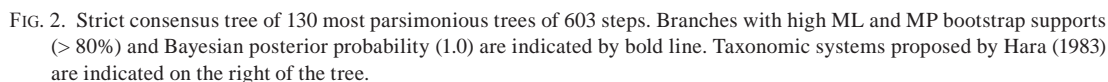


FIG. 1. Maximum likelihood tree of species of *Lonicera* based on chloroplast DNA sequences. Taxa newly examined are indicated in blue. Numbers above each branch are maximum likelihood bootstrap support / maximum parsimony bootstrap support / Bayesian posterior probability. Branches with high support values (> 80%) from maximum likelihood and maximum parsimony analyses and high posterior probability (1.0) from Bayesian analysis are indicated by bold lines. Less than 50% is indicated “-”.



Discussion

The present analyses indicated that our trees for *Lonicera* are fundamentally identical with those of Theis *et al.* (2008), and indicated that *Lonicera* is monophyletic and divided into two major clades corresponding to the two subgenera proposed by Rehder (1903, 1913), Hara (1983) and Hsu and Wang (1988). Moreover, monophyly of section *Lonicera* (= sect. *Coeloxylestem* sensu Rehder, see Table 1) and section *Isoxylestem* sensu Rehder is also supported by our trees. The results also support dividing section *Nintooa* into two clades (*L. calcarata* and the rest), while section *Ishika* is polyphyletic (Theis *et al.* 2008). Our analyses also provide further support the assignment of the Japanese species of *Lonicera* concerned here into subgenus *Lonicera* sensu Hara. Furthermore, our results add new insights for the placement of some endemic and native Japanese taxa and for determining the relationships among the Japanese species. Details are discussed below.

In the systems of Hara (1983) and Ohba (1993), section *Caeruleae*, characterized by fused ovaries and connate bracteoles, is represented by *Lonicera caerulea* (= *L. coerulea*). In our trees, samples of this section form a monophyletic group, suggesting that it is distant from other sections and subsections (Fig. 2, Table 1).

Section *Nintooa* is characterized by its climbing habit and twining or creeping stems and by flowers with a long, slender corolla tube (Hara 1983). According to the phylogenetic tree (Fig. 1), Japanese species of section *Nintooa* (*Lonicera japonica*, *L. miyagusukiana*, *L. affinis* and *L. hypoglauca*) form a monophyletic clade. *Lonicera miyagusukiana* has usually been recognized as a variety of *L. japonica* (H. Hara 1983, Ohba 1993). However, *L. miyagusukiana* differs remarkably from *L. japonica* in chloroplast DNA sequences and each forms a separate clade in the phylogenetic tree. Moreover, *L. japonica* is diploid ($2n=18$), whereas *L. miyagusukiana* is tetraploid ($2n=36$) (Denda *et al.* 2007), suggesting that *L. miyagusukiana* may be recognized as a distinct

species.

Section *Lonicera* is characterized by its shrubby habit, fistulose branches, and separated ovaries and berries. In this clade, we can find three major subclades with high branch support. One of them is a clade including *L. demissa* and *L. kurobushiensis*. Although these two species are distinguished from each other in degree of connation of the bracteoles, peduncle length, and shape of the calyx lobes (Kadota 2001), they do not show a differences in chloroplast DNA sequences, suggesting a close relationship between the two species. *Lonicera morrowii* was nested with *L. tatarica*, suggesting that it may be polyphyletic. Yang *et al.* (2011) treat *L. morrowii* as a variety of *L. tatarica*. These two species differ remarkably from each other in floral morphology; the flowers of *L. tatarica* are zygomorphic, while those of *L. morrowii* are actinomorphic (Hara 1983, Ohba 1993). To clarify their distinctness, further study with samples from various localities will be needed.

Section *Ishika* is characterized by bracteoles separate from each other (Hara 1983), but is diverse in the size and shape of the bracts and in the shape of the corolla tube. Hara (1983) subdivided the Japanese section *Ishika* into five subsections, *Purpurascetes* Rehder, *Monanthae* (Nakai)H. Hara, *Bracteatae* (Hook. f. *et* Thomos.)Rehder, *Ishika* and *Rhodanthae* (Maxim.) Rehder, on the basis of floral and vegetative characters. Our findings fundamentally support the monophyly of four of the subsections, except for *Purpurascetes*, proposed by Hara (1983), although the relationships among them remains unclear. The circumscription of the Japanese species of *Lonicera* proposed by Hara (1983) is fundamentally acceptable.

Concerning relationships among the subsections, it is notable that subsection *Rhodanthae* within section *Ishika* is more closely related sections *Nintooa* and *Lonicera* than to other subsections (Fig. 2). Within subsection *Rhodanthae*, *L. tschonoskii* of high elevations in central Honshu, forms the basal lineage and shows higher genetic differentiation than do other species, suggesting that it is distantly related to the other species of

TABLE 2. Character data of 5 loci on chloroplast DNA sequences.

Regions	Combined data	<i>atpB-rbcL</i>	<i>petN-psbM</i>	<i>psbM-trnD</i>	<i>rpoB-trnC</i>	<i>trnS-trnG</i>
Unaligned length		590 - 718	535 - 1224	601 - 1178	1074 - 1306	557 - 662
Aligned length	5663	748	1394	1241	1451	804
Length without gap positions	2463	514	292	281	988	388
Tree length	603	105	89	52	242	94
PI	280	60	28	16	88	44
CI	0.80	0.88	0.84	0.92	0.74	0.90
RI	0.91	0.96	0.89	0.93	0.82	0.95
Models	Proportional among loci	GTR + Γ	GTR + Γ	GTR + Γ	GTR + Γ	GTR + Γ

PI, number of parsimony informative sites; CI, consistency index; RI, retention index.

subsection *Rhodanthae*. Rehder (1903) suggested a close relationship of two Japanese species, *L. ramosissima* and *L. linderifolia*, with several Chinese species, such as *L. microphylla* and *L. tangutica* and assigned them to the same group within section *Purpurascences*. Our results indicate a position for these two Japanese species distant from the Chinese species of section *Purpurascences* (Figs. 1, 2). In the system proposed by Rehder (1903), *L. gracilipes* was assigned to subsection *Purpurascences*. Our study indicates that *L. gracilipes* forms an independent lineage and is distinct phylogenetically from the other members, such as the Chinese *L. microphylla* and *L. tangutica*. In subsection *Bracteatae*, *L. strophioophora*, endemic to Japan, is sister to *L. praeflorens*, a species widely distributed in eastern Asia, suggesting that they may be closely related. Because they form a monophyletic group in the phylogenetic tree, *L. harae* of Tsushima and Korea appears to be closely related to *L. fragrantissima* from China.

As discussed above, our investigations mainly focused on species from Japan, but suggest that some of them may be related to species in north-eastern Asia. To understand the relationships more clearly, detailed phylogenetic analyses that include species from northeastern Asia are needed.

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APPENDIX 1. List of taxa examined in this study with their geographical distribution, origin of plant samples, voucher specimens and accession numbers of the chloroplast DNA sequences.

Taxon	Distribution	Origin of samples	Voucher specimen	atpB-rbcL	petN-ps-bM	psbM-trnD	rpoB-trnC	trnS-trnG
<i>Lonicera acuminata</i> Wall.	SE Asia	1 Taiwan: Taichung County	<i>OKI 1040902</i>	AB937209	AB937293	AB937377	AB937461	AB937545
		2 Nepal: Gupha Bajar	<i>Ikeda 1226014</i>	AB937215	AB937299	AB937383	AB937467	AB937551
		3 Nepal: Gupha Bajar	<i>Ikeda 1224015</i>	AB937216	AB937300	AB937384	AB937468	AB937552
<i>L. affinis</i> Hook. & Arn.	Western Japan, endemic	1 Japan: Kagoshima, Amami Is.	<i>MAK 418213</i>	AB937140	AB937224	AB937308	AB937392	AB937476
		2 Japan: cult. at Makino Botanical Garden.	<i>m.n. 09042703</i>	AB937152	AB937236	AB937320	AB937404	AB937488
		3 Japan: Okinawa, Ogimison	<i>m.n. 10042401</i>	AB937191	AB937275	AB937359	AB937443	AB937527
		4 Japan: Okinawa, Nakijin	<i>m.n. 10042501</i>	AB937192	AB937276	AB937360	AB937444	AB937528
<i>L. alpigena</i> L. subsp. <i>alpigena</i>	Europe, NE Asia	1 USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265531	EU265595	EU265403	EU265467	EU265339
<i>L. alpigena</i> L. subsp. <i>glehnii</i> (F. Schmidt) Nakai	Japan, E Russia	2 Japan: cult. at Botanical Garden of Hokkaido Univ.	<i>MAK 418472</i>	AB937202	AB937286	AB937370	AB937454	AB937538
var. <i>glehnii</i>		3 Japan: Niigata, Tsunan	<i>m.n. 12060301</i>	AB937211	AB937295	AB937379	AB937463	AB937547
<i>L. alpigena</i> L. subsp. <i>glehnii</i> (F. Schmidt) Nakai var. <i>watanabeana</i> (Makino) H. Hara	Central Japan, endemic	4 Japan: Nagano, Ina	<i>m.n. 10051601</i>	AB937190	AB937274	AB937358	AB937442	AB937526
<i>L. bournei</i> Hemsl.	China, Myanmar	1 Myanmar: Shan	<i>m.n. 0092</i>	AB937148	AB937232	AB937316	AB937400	AB937484
<i>L. caerulea</i> L. subsp. <i>edulis</i> (Turcz.) Hultén var. <i>edulis</i>	Europe, Japan	1 Japan: cult. at Makino Botanical Garden	<i>m.n. 09042701</i>	AB937150	AB937234	AB937318	AB937402	AB937486
<i>L. caerulea</i> L. subsp. <i>eduli</i> (Turcz.) Hultén var. <i>emphylocalyx</i> (Maxim.) Nakai	Japan, Korea	2 Japan: cult. at Nikko Botanical Garden	<i>m.n. 09071604</i>	AB937168	AB937252	AB937336	AB937420	AB937504
<i>L. caerulea</i> L. subsp. <i>edulis</i> (Turcz.) Hultén var. <i>venulosa</i> (Maxim.) Rehder	Japan, (S Europe)	3 Japan: Iwate, Tono	<i>MAK 418473</i>	AB937208	AB937292	AB937376	AB937460	AB937544
<i>L. caerulea</i> L. (= <i>L. caerulea</i> Regel)	Europe	USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265535	EU265599	EU265407	EU265471	EU265343
<i>L. calcarata</i> Hemsl.	S China	China	<i>Theis et al. 2006</i>	EU265532	EU265596	EU265404	EU265468	EU265340
<i>L. carnosifolia</i> C.Y. Wu ex P. S. Hsu & H. J. Wang	China	China	<i>Theis et al. 2006</i>	EU265533	EU265597	EU265405	EU265469	EU265341
<i>L. caucasica</i> Pall.	W Asia	Japan: cult. at Nikko Botanical Garden	<i>m.n. 090716157</i>	AB937171	AB937255	AB937339	AB937423	AB937507
<i>L. cerasina</i> Maxim.	Japan, endemic	Japan: Kochi, Hongawa	<i>MAK 418474</i>	AB937188	AB937272	AB937356	AB937440	AB937524
<i>L. chrysantha</i> Turcz. ex Ledeb.	E Asia, Japan	USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265534	EU265598	EU265406	EU265470	EU265342
<i>L. conjugialis</i> Kellogg	W USA	Japan: cult. at Nikko Botanical Garden	<i>m.n. 09071618</i>	AB937173	AB937257	AB937341	AB937425	AB937509
<i>L. deflexicalyx</i> Batalin	SE China	USA: cult. at Quarryhill Botanical Garden	<i>Theis et al. 2006</i>	EU265536	EU265600	EU265408	EU265472	EU265344
<i>L. demissa</i> Rehder var. <i>borealis</i> H. Hara et M. Kichuchi	Japan, endemic	1 Japan: Iwate, Tono	<i>MAK 418475</i>	AB937205	AB937289	AB937373	AB937457	AB937541
		2 Japan: Iwate, Tono	<i>MAK 418476</i>	AB937206	AB937290	AB937374	AB937458	AB937542

APPENDIX 1 (CONTINUED)

Taxon	Distribution	Origin of samples	Voucher specimen	atpB-rbcL	petN-psbM	psbM-trnD	rpoB-trnC	trnS-trnG
<i>L. demissa</i> Rehder var. <i>demissa</i>	Japan, endemic	3 Japan: Nagano, Matsumoto	MAK 418214	AB937147	AB937231	AB937315	AB937399	AB937483
		4 Japan: Nagano, Matsumoto	<i>m.n.</i> 09052401	AB937162	AB937246	AB937330	AB937414	AB937498
		5 Japan: cult. at Nikko	<i>m.n.</i> 09071606	AB937169	AB937253	AB937337	AB937421	AB937505
		Botanical Garden	MAK 418213	AB937140	AB937224	AB937308	AB937392	AB937476
		6 Japan: Nagano, Chino	MAK 418477	AB937181	AB937265	AB937349	AB937433	AB937517
<i>L. dioica</i> L.	USA	USA: cult. at Smith College	<i>Theis et al.</i> 2006	EU265571	EU265635	EU265443	EU265507	EU265379
<i>L. etrusca</i> Santi.	W Asia, Europe	USA: cult. at Arnold Arboretum	<i>Theis et al.</i> 2006	EU265537	EU265601	EU265409	EU265473	EU265345
<i>L. ferdinandi</i> Franch	Korea, China	1 China	MAK 418478	AB937185	AB937269	AB937353	AB937437	AB937521
		2 USA: cult. at Arnold	<i>Theis et al.</i> 2006	EU265538	EU265602	EU265410	EU265474	EU265346
<i>L. giraldii</i> Rehder	China, SE Asia	USA: cult. at Arnold Arboretum	EU265552	EU265616	EU265424	EU265488	EU265360	AB937538
<i>L. gracilipes</i> Miq.	Japan, endemic	1 Japan	<i>Theis et al.</i> 2006	EU265540	EU265604	EU265412	EU265476	EU265348
<i>L. gracilipes</i> Miq. var. <i>glabra</i> Miq. f. <i>glabra</i>	Japan, endemic	2 Japan: Kyoto, Iwakura,	MAK 418479	AB937189	AB937273	AB937357	AB937441	AB937525
		3 Japan: Yamanashi, Tsuru	<i>m.n.</i> 10060203	AB937195	AB937279	AB937363	AB937447	AB937531
<i>L. gracilipes</i> Miq. var. <i>glandulosa</i> Maxim.	Japan, endemic	4 Japan: Yamanashi, Tsuru	MAK 418215	AB937146	AB937230	AB937314	AB937398	AB937482
<i>L. gracilipes</i> Miq. var. <i>gracilipes</i>	Japan, endemic	5 Japan: Tokyo, Hachioji	MAK 418480	AB937139	AB937223	AB937307	AB937391	AB937475
		6 Japan: Tokyo, Hinode	MAK 418481	AB937149	AB937233	AB937317	AB937401	AB937485
<i>L. graebneri</i> Rehder	SW China	China	<i>Theis et al.</i> 2006	EU265541	EU265605	EU265413	EU265477	EU265349
<i>L. gynochlamydea</i> Hemsl.	S China	1 USA: cult. at Arnold Arboretum	<i>Theis et al.</i> 2006	EU265542	EU265606	EU265414	EU265478	EU265350
		2 USA: cult. at Arnold Arboretum	<i>Theis et al.</i> 2006	EU265543	EU265607	EU265415	EU265479	EU265351
<i>L. harae</i> Makino	E Japan, Korea	1 South Korea: Jeju	MAK 418482	AB937153	AB937237	AB937321	AB937405	AB937489
		2 South Korea: Jeju	MAK 418483	AB937154	AB937238	AB937322	AB937406	AB937490
<i>L. hemsleyana</i> (Kuntze) Rehder	China	China	<i>Theis et al.</i> 2006	EU265544	EU265608	EU265416	EU265480	EU265387
<i>L. henryi</i> Hemsl.	Asia	USA: cult. at Arnold Arboretum	<i>Theis et al.</i> 2006	EU265545	EU265609	EU265417	EU265481	EU265353
<i>L. hispida</i> Pall.	SW China, S Asia	USA: cult. at Rancho Santa	<i>Theis et al.</i> 2006	EU265546	EU265610	EU265418	EU265482	EU265354
		Ana Botanical Garden						
<i>L. hypoglauca</i> Miq	SE Asia	Japan: cult. at Makino	<i>m.n.</i> 09072702	AB937151	AB937235	AB937319	AB937403	AB937487
		Botanical Garden						
<i>L. iberica</i> M. Bieb.	E Asia	USA: cult. at Arnol Arboretum	<i>Theis et al.</i> 2006	EU265548	EU265612	EU265420	EU265484	EU265356
<i>L. inconspicua</i> Batalin.	SW China	China	<i>Theis et al.</i> 2006	EU265549	EU265613	EU265421	EU265485	EU265457
<i>L. involucrata</i> Banks ex Spreng.	Europe, N America	USA: cult. at Arnold Arboretum	<i>Theis et al.</i> 2006	EU265550	EU265614	EU265422	EU265486	EU265358
<i>L. japonica</i> Thunb. var. <i>japonica</i> f. <i>japonica</i>	SE Asia, Japan	1 USA: cult. at Arnold Arboretum	<i>Theis et al.</i> 2006	EU265551	EU265615	EU265423	EU265487	EU265359
		2 Japan: Nagano, Matsumoto	<i>m.n.</i> 418217	AB937142	AB937226	AB937310	AB937394	AB937478
		3 South Korea: Busan	MAK 418484	AB937156	AB937240	AB937324	AB937408	AB937492
		4 Japan: cult. at Shimane University	<i>m.n.</i> 07052304	AB937160	AB937244	AB937328	AB937412	AB937496
		5 USA: Illinois, Carbonale	MAK 386935	AB937164	AB937248	AB937332	AB937416	AB937500
		6 Japan: Tokyo, Hachioji	<i>m.n.</i> 10052905	AB937194	AB937278	AB937362	AB937446	AB937530
<i>L. koehneana</i> Rehder	China	1 USA: cult. at Arnold Arboretum	<i>Theis et al.</i> 2006	EU265573	EU265637	EU265445	EU265509	EU265381
		2 USA: cult. at Arnold Arboretum	<i>Theis et al.</i> 2006	EU265577	EU265641	EU265449	EU265513	EU265385

APPENDIX 1 (CONTINUED)

Taxon	Distribution	Origin of samples	Voucher specimen	atpB-rbcL	petN-ps-bM	psbM-trnD	rpoB-trnC	trnS-trnG
<i>L. korolkowii</i> Stapf	Central Asia	China	MAK 418485	AB937187	AB937271	AB937355	AB937439	AB937523
<i>L. kurobushiensis</i> Kadota	Japan, endemic	1 Japan: Yamagata, Higashine	TNS 709742	AB937218	AB937302	AB937386	AB937470	AB937554
		2 Japan: Yamagata, Higashine	TNS 01151181	AB937219	AB937303	AB937387	AB937471	AB937555
		3 Japan: Yamagata, Higashine	TNS 01151182	AB937220	AB937304	AB937388	AB937472	AB937556
<i>L. linderifolia</i> Maxim. var. <i>konoi</i> (Makino) Okuyama	Japan, endemic	1 Japan: Nagano, Chino	MAK 418486	AB937179	AB937263	AB937347	AB937431	AB937515
		2 Japan: Nagano, Chino	MAK 418487	AB937180	AB937264	AB937348	AB937432	AB937516
<i>L. linderifolia</i> Maxim. var. <i>linderifolia</i>	Japan, endemic	3 Japan: Iwate, Kamaishi	m.n. 09092101	AB937175	AB937259	AB937343	AB937427	AB937511
		4 Japan: Iwate, Kamaishi	m.n. 10081501	AB937204	AB937288	AB937372	AB937456	AB937540
<i>L. maackii</i> (Rupr.) Maxim.	NE Asia, Japan	1 USA: cult. at Arnold Arboretum	Theis et al. 2006	EU265553	EU265617	EU265425	EU265489	EU265361
		2 Japan: Nagano, Karuizawa	MAK 418488	AB937159	AB937243	AB937327	AB937411	AB937495
		3 USA: Michigan, Ann Arbor	MAK 386936	AB937163	AB937247	AB937331	AB937415	AB937499
		4 Japan: cult. at Karuizawa Botanical Garden	m.n. 10060605	AB937198	AB937282	AB937366	AB937450	AB937534
		5 Russia: Ussuri. Cult. at Karuizawa Botanical Garden	m.n. 10060606	AB937199	AB937283	AB937367	AB937451	AB937535
		6 Japan: cult. at Koishikawa Botanical Garden	m.n. 11080801	AB937214	AB937298	AB937382	AB937466	AB937550
<i>L. maximowiczii</i> (Rupr.) Regel	NE Asia, Japan	USA: cult. at Arnold Arboretum	Theis et al. 2006	EU265554	EU265618	EU265426	EU265490	EU265362
<i>L. microphylla</i> Willd. ex Schult.	SW China, C Asia	China	MAK 418489	AB937186	AB937270	AB937354	AB937438	AB937522
<i>L. miyagusukiana</i> Makino	Japan, endemic	Japan: Okinawa, Iejima Is.	m.n. 11030842	AB937210	AB937294	AB937378	AB937462	AB937546
<i>L. mochidzukiana</i> Makino var. <i>mochidzukiana</i>	Japan, endemic	1 Japan: cult. at Nikko	m.n. 09071602	AB937166	AB937250	AB937334	AB937418	AB937502
		2 Botanical Garden Japan: Nikko Botanical Garden	m.n. 09071608	AB937170	AB937254	AB937338	AB937422	AB937506
<i>L. modesta</i> Rehder	China	USA: cult. at Arnold Arboretum	Theis et al. 2006	EU265574	EU265638	EU265446	EU265510	EU265382
<i>L. morrowii</i> A. Gray	E Asia, Japan	1 USA: cult. at Arnold Arboretum	Theis et al. 2006	EU265555	EU265619	EU265427	EU265491	EU265363
<i>L. morrowii</i> A. Gray f. <i>morrowii</i>		2 Japan: Nagano, Omachi	MAK 418490	AB937141	AB937225	AB937309	AB937393	AB937477
		3 Japan: Nagano, Matsumoto	MAK 418491	AB937144	AB937228	AB937312	AB937396	AB937480
		4 Japan: Iwate, Hachimantai	m.n. 09092304	AB937176	AB937260	AB937344	AB937428	AB937512
		5 Japan: cult. at Karuizawa Botanical Garden	m.n. 10060602	AB937197	AB937281	AB937365	AB937449	AB937533
		6 Japan: cult. at Nikko Botanical Garden	m.n. 09071603	AB937167	AB937251	AB937335	AB937419	AB937503
<i>L. myrtillos</i> Hook. f. & Thomson	SW China, S Asia	China	Theis et al. 2006	EU265556	EU265620	EU265428	EU265492	EU265364
<i>L. nervosa</i> Maxim.	W China	1 China	Theis et al. 2006	EU265558	EU265622	EU265430	EU265494	EU265366
		2 China	Theis et al. 2006	EU265557	EU265621	EU265429	EU265493	EU265365
<i>L. nigra</i> L.	Europe	-	Theis et al. 2006	EU265559	EU265623	EU265431	EU265495	EU265367
<i>L. nitida</i> E. H. Wilson	Europe	USA: cult. at Arnold Arboretum	Theis et al. 2006	EU265575	EU265639	EU265447	EU265511	EU265383
<i>L. orientalis</i> Lam.	Asia	USA: cult. at Arnold Arboretum	Theis et al. 2006	EU265576	EU265640	EU265448	EU265512	EU265384
<i>L. pileata</i> Oliv.	SW China	USA: cult. at Arnold Arboretum	Theis et al. 2006	EU265560	EU265624	EU265432	EU265496	EU265368
<i>L. praeflorens</i> Batalin var. <i>japonica</i> H. Hara	Japan, endemic	1 Japan: Nagano, Matsumoto	MAK 418218	AB937143	AB937227	AB937311	AB937395	AB937479
		2 Japan: Nagano, Matsumoto	m.n. 10060501	AB937196	AB937280	AB937364	AB937448	AB937532
<i>L. praeflorens</i> Batalin var. <i>praeflorens</i>	E Asia	3 South Korea: Gangwon	MAK 418492	AB937155	AB937239	AB937323	AB937407	AB937491

APPENDIX 1 (CONTINUED)

Taxon	Distribution	Origin of samples	Voucher specimen	atpB-rbcL	petN-psbM	psbM-trnD	rpoB-trnC	trnS-trnG
<i>L. pyrenaica</i> L.	Europe	USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265561	EU265625	EU265433	EU265497	EU265369
<i>L. ramosissima</i> Franch. et Sav.	Japan, endemic	1 Japan: Yamanashi, Tsuru	<i>MAK 418219</i>	AB937145	AB937229	AB937313	AB937397	AB937481
ex Maxim. var. <i>ramosissima</i>		2 Japan: Yamagata. cult. at Tokyo Met. Univ.	<i>m.n. 10052901</i>	AB937193	AB937277	AB937361	AB937445	AB937529
f. <i>glabrata</i> (Nakai) H. Hara		3 Japan: cult. at Karuizawa Botanical Garden	<i>m.n. 10060607</i>	AB937200	AB937284	AB937368	AB937452	AB937536
		4 Japan: Niigata, Agamachi	<i>MAK 418493</i>	AB937203	AB937287	AB937371	AB937455	AB937539
		5 Japan: Yamanashi, Tsuru	<i>m.n. 10061002</i>	AB937157	AB937241	AB937325	AB937409	AB937493
<i>L. ramosissima</i> Franch. et Sav.	Japan, endemic	6 Japan: Kanagawa, Hakone, cult. at Tokyo Met. Univ.	<i>m.n. 09090401</i>	AB937174	AB937258	AB937342	AB937426	AB937510
ex Maxim. var. <i>ramosissima</i> f. <i>ramosissima</i>								
<i>L. ruprechtiana</i> Regel	NE China	USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265578	EU265642	EU265450	EU265514	EU265386
<i>L. sovetkinae</i> L.	-	USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265564	EU265628	EU265436	EU265500	EU265372
<i>L. spinosa</i> Jacq.	SW China, S Asia	Tibet	<i>Theis et al. 2006</i>	EU265565	EU265629	EU265437	EU265501	EU265373
<i>L. standishii</i> Jacq.	China	USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265566	EU265630	EU265438	EU265502	EU265374
<i>L. strophiphora</i> Franch.	Japan, endemic	1 Japan: Iwate, Tono	<i>MAK 418494</i>	AB937207	AB937291	AB937375	AB937459	AB937543
var. <i>strophiphora</i> f. <i>glabrifolia</i> (Ohwi) H. Hara								
<i>L. strophiphora</i> Franch.		2 Japan: cult. at Nikko Botanical Garden	<i>m.n. 09071616</i>	AB937172	AB937256	AB937340	AB937424	AB937508
var. <i>strophiphora</i> f. <i>strophiphora</i>		3 Japan: Yamanashi, Tsuru	<i>m.n. 10060202</i>	AB937158	AB937242	AB937326	AB937410	AB937494
<i>L. subsessilis</i> Rehder	E Asia	USA: cult. at Arnold Arboretum Botanical Garden	<i>Theis et al. 2006</i>	EU265579	EU265643	EU265451	EU265515	EU265387
<i>L. tangutica</i> Maxim.	SW China	China	<i>Theis et al. 2006</i>	EU265567	EU265631	EU265439	EU265503	EU265375
<i>L. tatarica</i> L.	NE Asia	1 USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265568	EU265632	EU265440	EU265504	EU265376
		2 USA: Michigan	<i>m.n. 09062201</i>	AB937165	AB937249	AB937333	AB937417	AB937501
		3 China	<i>MAK 418495</i>	AB937184	AB937268	AB937352	AB937436	AB937520
<i>L. tragophylla</i> Hemsl.	China	China	<i>Theis et al. 2006</i>	EU265569	EU265633	EU265441	EU265505	EU265377
<i>L. tschonoskii</i> Maxim.	Japan, endemic	Japan: Nagano, Matsumoto	<i>m.n. 09082301</i>	AB937212	AB937296	AB937380	AB937464	AB937548
<i>L. uzenensis</i> Kadota	Japan, endemic	1 Japan: Yamagata, Takahata	<i>TNS 710812</i>	AB937221	AB937305	AB937389	AB937473	AB937557
		2 Japan: Yamagata, Takahata	<i>TNS 710815</i>	AB937222	AB937306	AB937390	AB937474	AB937558
<i>L. vesicaria</i> Kom.	Korea, NE China	USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265570	EU265634	EU265442	EU265506	EU265378
<i>L. vidalii</i> Franch. et Sav.	Japan, Korea	1 Japan: Shimane, Oki	<i>m.n. 07102012</i>	AB937161	AB937245	AB937329	AB937413	AB937497
		2 Japan: cult. at Karuizawa Botanical Garden	<i>m.n. 09100303</i>	AB937177	AB937261	AB937345	AB937429	AB937513
		3 Japan: cult. at Karuizawa Botanical Garden	<i>m.n. 09100304</i>	AB937178	AB937262	AB937346	AB937430	AB937514
		4 Japan: Nagano, Karuizawa	<i>m.n. 10060608</i>	AB937201	AB937285	AB937369	AB937453	AB937537
<i>L. xylosteum</i> L.	Europe	1 USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265572	EU265636	EU265444	EU265508	EU265380
		2 USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265581	EU265645	EU265453	EU265517	EU265389
<i>Leycesteria crocothyrsos</i> Airy Shaw	SW China	UK: cult. at Kew Gardens	<i>Theis et al. 2006</i>	EU265520	EU265584	EU265392	EU265456	EU265328
<i>L. formosa</i> Wall.	S China, S Asia	1 China	<i>Theis et al. 2006</i>	EU265521	EU265585	EU265393	EU265457	EU265329
		2 Nepal: Gupha Bajar	<i>Ikeda 1226031</i>	AB937217	AB937301	AB937385	AB937469	AB937553

APPENDIX 1 (CONTINUED)

Taxon	Distribution	Origin of samples	Voucher specimen	atpB-rbcL	petN-ps-bM	psbM-trnD	rpoB-trnC	trnS-trnG
<i>Symphoricarpos</i>	N America	USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265522	EU265586	EU265394	EU265458	EU265330
<i>hesperius</i> G. N. Jones								
<i>S. occidentalis</i> Hook.	N America	USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265523	EU265587	EU265395	EU265459	EU265331
<i>S. orbiculatus</i> A. Gray	N America	USA: cult. at Arnold Arboretum	<i>Theis et al. 2006</i>	EU265524	EU265688	EU265396	EU265460	EU265332
<i>Triosteum angustifolium</i> L.	N America	USA: Ohio Univ. Herbarium	<i>Theis et al. 2006</i>	EU265525	EU265589	EU265397	EU265461	EU265333
<i>T. aurantiacum</i> E. P. Bicknell	N America	USA: Ohio Univ. Herbarium	<i>Theis et al. 2006</i>	EU265526	EU265590	EU265398	EU265462	EU265334
<i>T. perfoliatum</i> L.	N America	USA: Ohio Univ. Herbarium	<i>Theis et al. 2006</i>	EU265527	EU265591	EU265399	EU265463	EU265335

SN: Sample number, BG: Botanical Garden, C: Central, N: North, S: South, E: East, W: West.